



## **The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017**

**Karlslose Pedersen, Susanne; Frimann, Jens-Ole Marinus; Aarestrup, Frank Møller; Hendriksen, Rene S.**

*Publication date:*  
2019

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Karlslose Pedersen, S., Frimann, J-O. M., Aarestrup, F. M., & Hendriksen, R. S. (2019). *The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017*. Technical University of Denmark.

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# The External Quality Assurance System of the WHO Global Foodborne Infections Network, 2017



World Health  
Organization



# **THE EXTERNAL QUALITY ASSURANCE SYSTEM OF THE WHO GLOBAL FOODBORNE INFECTIONS NETWORK YEAR 2017**

**Susanne Karlsmosse Pedersen, Jens-Ole Frimann, Frank M. Aarestrup, Rene S. Hendriksen**

1. edition, January 2019

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Photo: Mikkel Adsbøl

ISBN: 978-87-93565-38-8

The report is available at

[www.food.dtu.dk](http://www.food.dtu.dk)

National Food Institute

Technical University of Denmark

Kemitorvet

Building 204

DK-2800 Kgs. Lyngby

Denmark

Tel: +45 35 88 70 00

Fax +45 35 88 70 01

## **List of Abbreviations**

**AGISAR**, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance

**AMR**, Antimicrobial Resistance

**AST**, Antimicrobial Susceptibility Testing

**ATCC**, American Type Culture Collection

**CAZ**, Ceftazidime

**CDC**, Centers for Disease Control and Prevention

**COL**, Colistin

**CRO**, Ceftriaxone

**CTX**, Cefotaxime

**DTU Food**, Technical University of Denmark, National Food Institute

**EQAS**, External Quality Assurance System

**ESBL**, Extended Spectrum Beta-Lactamase

**GMI**, Global Microbial Identifier

**IP**, Institute Pasteur

**MERO**, Meropenem

**MIC**, Minimum Inhibitory Concentration

**SMX**, Sulfamethoxazole

**SXT**, Sulfamethoxazole-trimethoprim (co-trimoxazole)

**WHO**, World Health Organization

**WHO GFN**, WHO Global Foodborne Infections Network

## 1. Introduction

Since 2000, 16 WHO External Quality Assurance System (EQAS) reports have been issued with this report being the 17<sup>th</sup>. The WHO Global Foodborne Infections Network (WHO GFN) and the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) focus on enhancing World Health Organization (WHO) Member States' capacity to detect and respond to foodborne disease outbreaks and the emerging of antimicrobial resistance (AMR) in bacterial pathogens by conducting laboratory-based surveillance of *Salmonella* and other important foodborne pathogens. Thus, the WHO EQAS 2017 aligns with the 2015 WHO global action plan to target AMR worldwide, objective 2: Strengthen knowledge through surveillance and research, action 2, laboratory capacity.

Since its inception, the scope of the WHO EQAS has expanded to include additional foodborne pathogens than *Salmonella* such as *Shigella* and *Campylobacter*. *Salmonella*, *Campylobacter* and *Shigella* are among the most important foodborne pathogens worldwide and accounts for millions of cases of diarrheal disease and thousands of deaths per year impacting both developing and industrialized countries. Furthermore, the increased number of *Salmonella*, *Campylobacter* and *Shigella* isolates which are resistant to antimicrobials is of major concern since these bacterial isolates are associated with infections characterized by increased morbidity and mortality.

In the 2017 iteration of the WHO EQAS, a major change was applied as it focuses only on *Salmonella* serotyping and antimicrobial susceptibility testing (AST). This adjustment was made to balance the costs and focus efforts at continuing the development of the genomic proficiency test adopted by WHO and offered through the Global Microbial Identifier (GMI) (<http://www.globalmicrobialidentifier.org/workgroups/about-the-gmi-proficiency-tests>).

The WHO EQAS is organized annually by DTU Food in collaboration with World Health Organization (WHO) in Geneva, Switzerland, Centers for Disease Control and Prevention (CDC) in Atlanta, USA, and Institute Pasteur (IP) in Paris, France.

Individual laboratory data are confidential and known only by the participating laboratory, the EQAS Organizer (DTU Food) and possibly the respective WHO GFN regional centre/WHO AGISAR country representative. All summary conclusions are public. The goal set by WHO GFN/AGISAR aims at having all national reference laboratories perform *Salmonella* serotyping with a maximum of one deviation out of eight strains tested (error rate of 13%) and performing AST of *Salmonella* with a maximum error rate of 10% (either less than 5% very major / major errors and less than 5% minor errors, or less than 10% minor errors). Minor deviations are defined as classification of an intermediate strain as susceptible, resistant or vice versa (*i.e.*  $I \leftrightarrow S$  or  $I \leftrightarrow R$ ). Major deviation is the classification of a susceptible strain as resistant (*i.e.*  $S \rightarrow R$ ). Very major deviation is the classification of a resistant strain as susceptible (*i.e.*  $R \rightarrow S$ ). In this report, the deviations of AST results are divided into two categories, *i.e.* critical deviations which include major and very major deviations, and total deviations which include also the minor deviations. In EQAS 2014, the regions were re-defined for all countries worldwide in relation to the analysis of data from the WHO GFN EQAS. This resulted in some reorganization of countries into new regions compared to previous years, why interpretation of regional-based results from 2014 and onwards

with results from before 2014 should be conducted with care. The countries belonging to each region is listed in Appendix 1.

Appendices 2-5 present additional background information in relation to the WHO EQAS 2017.

## 2. Summary

The summary report is divided into sections; the serotyping component, AMR as well as reporting resistance to Extended Spectrum Beta-Lactamases (ESBL) producing *Salmonella*. All results reported in the summary can be found in Appendix 1.

### Participation

A total of 191 laboratories responded to the pre-notification and were enrolled in the WHO EQAS. When the deadline for submitting results was reached, 181 laboratories in 81 countries had uploaded data.

The following countries provided data for at least one of the EQAS components (Appendix 1): Argentina, Australia (3), Bahrain, Bangladesh, Barbados, Belgium, Belize, Bolivia, Brazil (2), Brunei Darussalam, Bulgaria, Cambodia, Cameroon, Canada (11), Chile, China (15), Colombia (4), Costa Rica (2), Croatia, Cuba, Curaçao, Cyprus, Czech Republic (2), Denmark, Ecuador, Egypt, Gambia, Germany (2), Ghana, Greece (2), Guatemala (2), Honduras, India (4), Iran, Islamic rep. of (3), Ireland, Israel, Italy (14), Jamaica, Japan (2), Kenya (2), Korea, Rep of (2), Kosovo, Lao PDR, Luxembourg (2), Madagascar, Malaysia (5), Malta, Mauritius, Mexico (3), Morocco, Nepal (6), New Zealand, Nigeria (4), Panama (2), Paraguay, Peru, Philippines (2), Poland (3), Portugal, Serbia (2), Singapore (2), Slovakia, Slovenia, South Africa, Spain, Sri Lanka (2), Suriname, Sweden, Taiwan, Tanzania, United Republic of, Thailand (16), Trinidad and Tobago, Turkey, Ukraine, United Kingdom, United States of America (5), Uruguay, Venezuela (2), Viet Nam (2), Zambia, and Zimbabwe.

The level of participation in the WHO EQAS 2017 was the same as at the WHO EQAS 2016.

### *Salmonella* EQAS components

The acceptance threshold for the EQAS *Salmonella* serotyping component was met by 77% (n = 111) of the 145 participating laboratories (Table 1). In addition, 88% (n = 127) of the laboratories tested all eight strains with a total of 90% (n = 1.014) of all tests being correct, representing results almost at the same level as in 2016 which was one of the best performances observed since the initiation of the EQAS (Table 2). The ability to correctly serotype the internal control strain increased in 2017 to the same level as in 2014, 98%, which is the best performance, recorded and only observed previously in 2011 and 2014. The increase in performance observed compared to

2016 was most likely due to a lower number of participating laboratories serotyping this specific strain. In 2017, the participation in testing the internal control strain decreased from 159 to 142, a level previously recorded over the years (Table 3). On a region-based categorization of participating laboratories, Africa and the Central Asia & Middle East both correctly serotyped between 63% and 66% of the test strains whereas the Caribbean, Southeast Asia, and Latin America, correctly serotyped between 81% and 89% of the test strains. The performance of correct serotyping in Europe, China, North America was between 94 and 99% but reached 100% correct serotyping of all eight strains in only Oceania. In 2017, Russia was again the only region not participating (Table 4). In all regions except for the Central Asia & Middle East region either a marked or consistent improvement was observed and in line with the other data presented.

In 2017, the main problem regarding the *Salmonella* serotyping appeared relatively to be associated with strain, WHO 2017 S-17.8 (Kentucky) whereas the deviations for the rest of the strains seems to be acceptable at a level of approximately 10% (n=5) and for the remaining two strains at 6% and 2%.

As indicated, WHO 2017 S-17.8 (Kentucky, I 8,20:i:z6), revealed a considerable level of deviation at 17.0% (Table 5). Of the 23 deviations, 14 were attributed to Tumodi (I 1,4,12:i:z6) which only differs from the somatic O antigen compared to Kentucky. It is surprising that the problem of the serotyping procedure seems very often to be associated with the somatic O antigen of relatively common antigens. The level of deviation is surprising since the serovars included the 2017 should not pose major difficulties. The somatic O antigens of all the test strains belong to the major serogroups such as O:4, O:3,10, O:7, O:8, and O:9, and the flagella antigens belong to well-known polyvalent antisera complex G and HMD.

Concerning the *Salmonella* AST component for the EQAS 2017, the performance slightly decreased compared to the EQAS of 2016, with deviations of 3% minor, 2% major, and 3% very major deviations. Thus, the percentages of critical deviation was 5% (Table 6). Deviations categorized by the tested antimicrobials revealed that ceftazidime (CAZ), ciprofloxacin (CIP), colistin (COL), ceftriaxone (CRO), cefotaxime (CTX), meropenem (MERO), sulfamethoxazole (SMX) and co-trimoxazole (SXT) caused most of the difficulties observed with the following level of total deviations: 22%, 18%, 6%, 7%, 8%, 6%, 7%, and 7%, respectively (Table 7). The deviations to CIP was mostly attributed to minor deviations and most likely due to the often observed hazy double zone when performing disk diffusion where the outer zone often incorrectly is measured. In this year's iteration, participating laboratories appears to have been too strict measuring the zone diameter categorizing the susceptible strains intermediate. Similarly, the deviations observed to SMX and SXT are due to the bacteriostatic effect complicating reading when conducting both disk diffusion and minimum inhibitory concentration (MIC) determination where 20% of the lawn of growth for disk diffusion equal to 80% reduction of growth for MIC determination determines the read-value. This year, a resistant isolate caused most problems. For the disk diffusion results, it was not surprising to see deviations in relation to COL as disk diffusion is not recommended as a method for AST to colistin. This resulted in 10 participants incorrectly reporting one isolate susceptible despite it being resistant. For the four antimicrobials used to confirm ESBL and carbapenemase production, CAZ, CRO, CTX and MERO, all were responsible

for critical deviations with 17% of all tests incorrect for CAZ, which is a great concern (Table 7, Table 8). Assessing the data for the four antimicrobials, no clear patterns was observed, resistance reported as susceptible and visa versa (Table 8).

On a region-based categorization of participating laboratories, all regions performed poorly compared to 2016. A greater number of deviations was observed in developing regions, which partly could explain the results as well as the difficulties reporting the results for the third generation cephalosporins. The Caribbean region obtained the highest percentages of total deviations, 24.3% whereas a number of regions obtained a total deviations around 10%, *i.e.* Africa (12.8%), China (6.6%), Southeast Asia (8.1%), Latin America (8.9%), Europe (7.2%), and Central Asia & Middle East (11.1%). None of the regions obtained a performance of 100% correct AST results, however, North America and Oceania performed better than the other regions with 97.1% and 96.1% correct AST-results. Russia did not participate in the 2017 EQAS (Table 9).

For the 150 laboratories performing the *Salmonella* AST component (MIC (n = 41)/Disk diffusion (n = 74)), only 77% (115 laboratories) reported data for AST of the control strain *E. coli* ATCC 25922. As in previous years, this is a very alerting number as it is expected that all participating laboratories follow quality assurance procedures (Table 10). It is of extreme importance to once again emphasize that this component represents the true indicator for the laboratory as to the performance of AST. It is noteworthy that the WHO EQAS organizers provide the control strain *E. coli* ATCC 25922 free of charge to all new participants of the AST component, why there should not be any excuses not to test this strain.

### **ESBL EQAS component**

The participants of the AST component are offered to detect and confirm ESBL production in the *Salmonella* strains. If participating in this component of the EQAS, all strains showing reduced susceptibility to cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ) ceftriaxone (CRO) and/or meropenem (MERO) should be tested for ESBL, AmpC and carbapenemase production.

For the EQAS 2017, four AmpC-, ESBL-, carbapenemase-producers were included represented by WHO 2017 S-17.1 Infantis (ESBL), WHO 2017 S-17.2 Havana (AmpC), WHO 2017 S-17.4 Rissen (ESBL), and WHO 2017 S-17.8 Kentucky (carbapenemase producers) (Table 11). The two ESBL producing strains harboured the *bla*<sub>CTX-M14b</sub>, and *bla*<sub>CTX-M14</sub> genes whereas the gene accounting for the AmpC phenotype till now curiously is unknown. The carbapenemase producer was conferred by *bla*<sub>NDM-1</sub> and *bla*<sub>CMY-16</sub>. The confirmatory tests (CAZ/Cl:CAZ and CTX/Cl:CTX) showed 87% (WHO 2017 S-17.1) and 90% (WHO 2017 S-17.4) of deviations in reporting correct ESBL results (based on phenotypic characteristics). For the WHO 2017 S-17.2 (AmpC) and WHO 2017 S-17.4 (carba), deviations of the confirmatory test resulted in 66% and 34%. In general, the level of correctly identified ESBL, AmpC and carbapenemase producing *Salmonella* is a great concern and it is suggested that the participating laboratories take steps to ensure that it is improved.



### **3. List of Appendices**

Appendix 1: Figures and Tables

Appendix 2: Prenotification

Appendix 3: Expected results

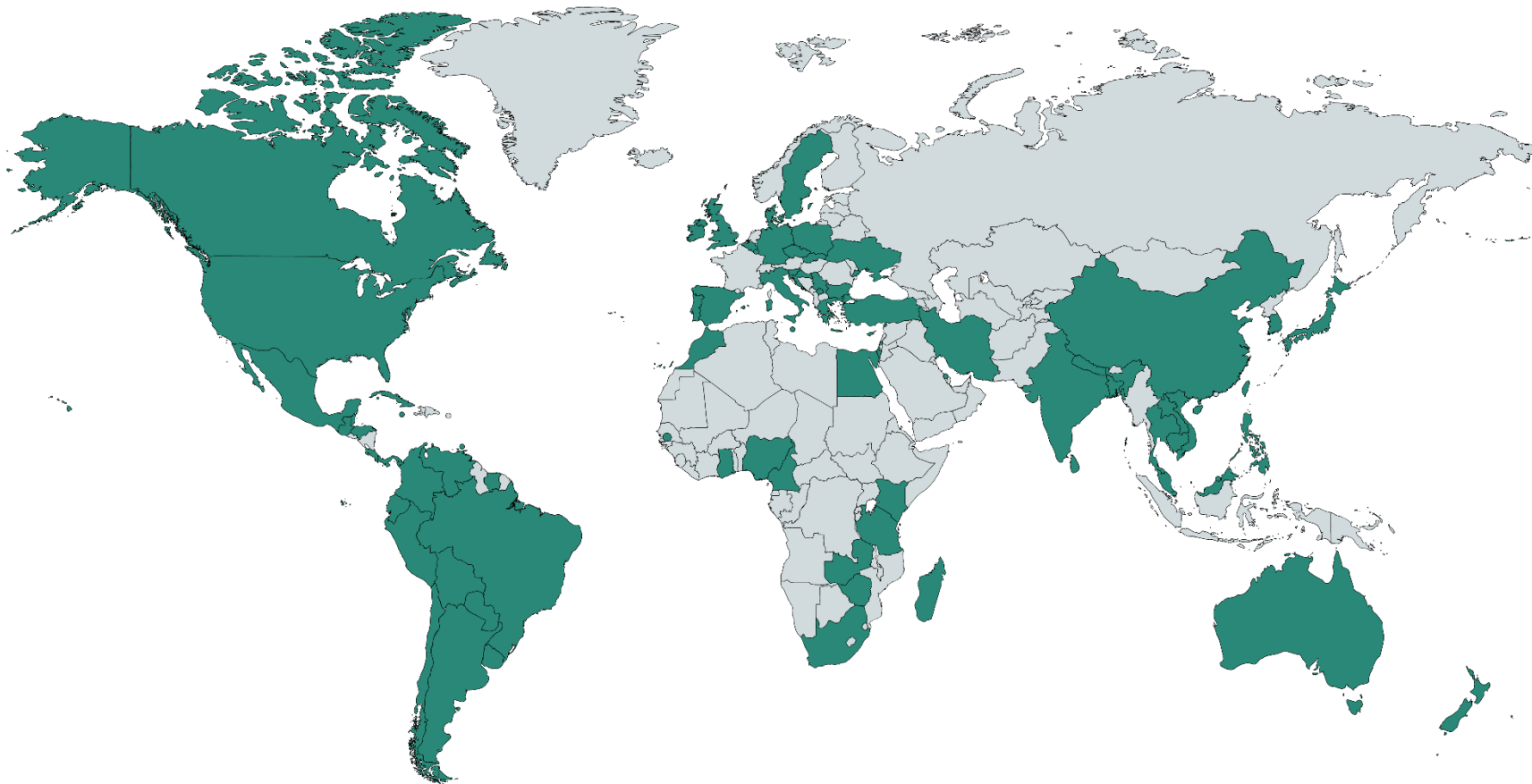
Appendix 4: WHO EQAS 2017 Protocol

Appendix 5a: Subculture and Maintenance of Quality Control Strains

Appendix 5b: Instructions for Opening and Reviving Lyophilized Cultures

## Figure and Tables

Figure 1. Countries participating\* in the WHO EQAS 2017



Created with mapchart.net ©

\*marked in green

## List of Countries in the 10 Regions

### Africa

Algeria	Gabon	Reunion
Angola	Gambia	Rwanda
Benin	Ghana	Saint Helena
Botswana	Guinea	Sao Tome and Principe
Burkina Faso	Guinea-Bissau	Senegal
Burundi	Kenya	Seychelles
Cameroon	Lesotho	Sierra Leone
Cameroun	Liberia	Somalia
Cape Verde	Libyan Arab Jamahiriya	South Africa
Central African Republic	Madagascar	South Sudan
Chad	Malawi	Sudan
Comoros	Mali	Swaziland
Congo (Brazzaville)	Mauritania	Tanzania, United Republic of
Congo, Democratic Republic of the	Mauritius	Togo
Cote d'Ivoire (Ivory Coast)	Mayotte	Tunisia
Djibouti	Morocco	Uganda
Egypt	Mozambique	Western Sahara
Equatorial Guinea	Namibia	Zambia
Eritrea	Niger	Zimbabwe
Ethiopia	Nigeria	

### Caribbean

Anguilla	Dominica	Saint Martin
Antigua and Barbuda	Dominican Republic	Saint Vincent and the Grenadines
Aruba	Grenada	Saint-Barthélemy
Bahamas	Guadeloupe	Sint Maarten
Barbados	Haiti	St. Kitts and Nevis
Bonaire, Saint Eustatius and Saba	Jamaica	Trinidad and Tobago
British Virgin Islands	Martinique	Turks and Caicos Islands
Cayman Islands	Monserrat	Virgin Islands (US)
Cuba	Puerto Rico	
Curaçao	Saint Lucia	

### Central Asia & Middle East

Afghanistan	Israel	Pakistan
Armenia	Jordan	Palestine
Azerbaijan	Kazakhstan	Qatar
Bahrain	Kuwait	Saudi Arabia
Bangladesh	Kyrgyzstan	Syria
Bhutan	Lebanon	Tajikistan
Georgia	Macao	Timor Leste (West)
Hong Kong	Maldives	Turkmenistan
India	Mongolia	United Arab Emirates
Indonesia	Myanmar (ex-Burma)	Uzbekistan
Iran, Islamic rep. Of	Nepal	Yemen
Iraq	Oman	

### China

China

### Europe

Albania	Guernsey and Alderney	Norway
Andorra	Hungary	Poland
Austria	Iceland	Portugal
Belarus	Ireland	Romania

Belgium  
Bosnia  
Bulgaria  
Croatia  
Cyprus  
Czech Republic  
Denmark  
Estonia  
European Union  
Faroe Islands  
Finland  
France  
Germany  
Gibraltar  
Greece

Italy  
Jersey  
Kosovo  
Latvia  
Liechtenstein  
Lithuania  
Luxembourg  
Macedonia  
Malta  
Man, Island of  
Moldova  
Monaco  
Montenegro  
Netherlands

San Marino  
Serbia  
Slovak Republic  
Slovakia  
Slovenia  
Spain  
Svalbard and Jan Mayen Islands  
Sweden  
Switzerland  
Turkey  
Ukraine  
United Kingdom  
Vatican City State (Holy See)

### **Latin America**

Argentina  
Bolivia  
Brazil  
Chile  
Colombia  
Costa Rica  
Ecuador

El Salvador  
Falkland Islands (Malvinas)  
French Guiana  
Guatemala  
Guyana  
Honduras  
Mexico

Nicaragua  
Panama  
Paraguay  
Peru  
Suriname  
Uruguay  
Venezuela

### **North America**

Bermuda  
Canada

Greenland  
Saint Pierre and Miquelon

United States of America

### **Oceania**

Australia  
Kiribati  
New Zealand  
Solomon, Islands  
Fiji  
Marshall Islands

Papua New Guinea  
Tonga  
French Polynesia  
Micronesia  
Samoa  
Tuvalu

Guam  
New Caledonia  
Samoa, American  
Vanuatu

### **Russia**

Russia

### **Southeast Asia**

Brunei Darussalam  
Cambodia  
Japan  
Korea, North  
Korea, Rep of

Lao PDR  
Malaysia  
Philippines  
Singapore  
Sri Lanka

Taiwan  
Thailand  
Viet Nam

Table 1. Ability of EQAS participating laboratories to serotype the test *Salmonella* strains

Number of strains correctly serotyped	Participating laboratories													
	EQAS 2000		EQAS 2001		EQAS 2002		EQAS 2003		EQAS 2004		EQAS 2006		EQAS 2007	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	9	24	34	35	52	53	66	47	41	32	42	32	66	47
7	9	24	13	14	19	19	29	21	14	11	35	27	29	21
6	4	11	9	9	12	12	13	9	16	13	19	15	13	9
5	3	8	9	9	4	4	11	8	16	13	12	9	11	8
4	3	8	4	4	1	1	7	5	11	9	7	5	7	5
3	4	11	8	8	4	4	6	4	10	8	5	4	6	4
2	2	5	3	3	5	5	2	1	10	8	3	2	2	1
1	2	5	5	5	1	1	6	4	5	4	4	3	6	4
0	1	3	11	11	1	1	0	0	4	3	3	2	0	0
In total	37	100	96	100	99	100	127	100	127	100	130	100	140	100
	Participating laboratories													
	EQAS 2008		EQAS 2009		EQAS 2010		EQAS 2011		EQAS 2012		EQAS 2013		EQAS 2014	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
8	50	33	76	50	91	61	82	67	68	47	52	41	70	47
7	36	24	29	19	16	11	17	14	29	20	29	23	32	21
6	11	7	7	5	12	8	10	8	14	10	15	12	17	11
5	14	9	13	8	9	6	2	2	9	6	8	6	6	4
4	12	8	5	3	6	5	4	3	5	3	7	6	5	3
3	9	6	7	5	2	1	4	3	6	4	7	6	7	5
2	8	6	5	3	2	1	1	1	10	7	6	5	4	3
1	9	6	6	4	7	5	3	2	2	1	2	2	4	3
0	2	1	5	3	3	2	0	0	1	1	0	0	4	3
In total	151	100	153	100	148	100	123	100	144	100	126	100	149	100
	Participating laboratories													
	EQAS 2015		EQAS 2016		EQAS 2017		Average EQAS 2000 - 2017							
	No.	%	No.	%	No.	%	No.	%						
8	65	43	84	58	85	59	61	46						
7	25	17	22	15	26	18	24	19						
6	17	11	18	12	14	10	13	10						
5	22	15	5	3	7	5	9	7						
4	5	3	5	3	5	3	6	5						
3	2	1	5	3	3	2	6	5						
2	4	3	3	2	0	0	4	3						
1	7	5	4	3	4	3	5	4						
0	4	3	0	0	1	1	2	2						
In total	151	100	146	100	145	100	129	100						

Table 2. EQAS participating laboratories' performance of *Salmonella* serotyping

EQAS iteration	Labs serotyping all provided strains		Correct test results	
	No.	%	No.	%
2000	34	92	165	76
2001	79	82	513	72
2002	80	81	668	91
2003	69	54	692	80
2004	78	61	701	81
2006	105	81	808	85
2007	109	78	920	88
2008	100	66	888	83
2009	119	83	974	86
2010	129	87	998	89
2011	109	89	878	92
2012	122	81	936	83
2013	74	59	812	89
2014	85	57	969	92
2015	104	69	948	87
2016	130	89	1004	90
2017	127	88	1014	90
<b>Average</b>	<b>101</b>	<b>76</b>	<b>817</b>	<b>86</b>

Table 3. EQAS participating laboratories' performance of internal quality control strain (WHO S-17.3, *Salmonella* Enteritidis) serotyping).

EQAS iteration	Labs serotyping <i>S. Enteritidis</i> correctly	
	No.	%
2000	34	92
2001	64	84
2004	113	95
2006	116	94
2007	135	96
2008	139	96
2009	141	93
2010	138	97
2011	128	98
2012	139	96
2013	130	96
2014	145	98
2015	125	93
2016	159	89
2017	142	98
<b>Average</b>	<b>123</b>	<b>94</b>

Table 4. Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2017
Africa	2001	6	37	73.0	Cameroun, Egypt, Kenya, Madagascar, Mauritius, Morocco, Nigeria (2), South Africa
	2002	9	62	87.1	
	2003	11	70	71.4	
	2004	9	51	62.7	
	2006	16	95	71.6	
	2007	11	73	80.8	
	2008	10	71	49.3	
	2009	15	94	75.5	
	2010	13	83	67.5	
	2011	10	57	79.2	
	2012	10	65	60.0	
	2013	8	51	74.5	
	2014	11	63	76.2	
	2015	12	68	61.8	
	2016	8	58	62.1	
	<b>2017</b>	<b>9</b>	<b>56</b>	<b>62.5</b>	
Central Asia & Middle East	2001	10	60	50.0	Bahrain, India, Israel, Nepal
	2002	5	30	83.3	
	2003	5	35	54.3	
	2004	5	33	54.5	
	2006	5	35	74.3	
	2007	5	40	55.0	
	2008	5	34	61.8	
	2009	5	32	46.9	
	2010	5	22	75.9	
	2011	3	23	95.8	
	2012	4	30	56.7	
	2013	5	38	52.6	
	2014	7	37	75.7	
	2015	7	44	77.3	
	2016	5	38	78.9	
	<b>2017</b>	<b>4</b>	<b>32</b>	<b>65.6</b>	
Caribbean	2001	0	0	0	Barbados, Cuba, Curaçao, Trinidad and Tobago
	2002	0	0	0	
	2003	3	18	61.1	
	2004	2	8	87.5	
	2006	3	14	78.6	
	2007	2	9	77.8	
	2008	3	14	78.6	
	2009	3	12	83.3	
	2010	2	13	92.9	
	2011	1	7	87.5	
	2012	2	16	62.5	
	2013	1	5	100.0	
	2014	3	15	60.0	
	2015	5	24	58.3	
	2016	2	16	60	
	<b>2017</b>	<b>4</b>	<b>32</b>	<b>81.3</b>	
Europe	2001	43	323	80.5	Belgium, Bulgaria, Croatia, Cyprus, Czech Republic (2), Denmark, Germany (2), Greece (3), Ireland, Italy (14), Luxembourg (2), Poland (3), Portugal, Serbia (2), Slovak Republic, Slovenia, Spain, Sweden, Turkey, Ukraine, United Kingdom
	2002	50	384	90.0	
	2003	60	401	84.8	
	2004	57	392	84.7	
	2006	52	403	86.4	
	2007	54	415	89.4	
	2008	50	379	82.3	
	2009	47	362	93.1	
	2010	45	332	94.1	
	2011	42	314	94.6	
	2012	47	368	92.9	
	2013	42	309	94.5	
	2014	52	391	96.2	
	2015	48	371	93.8	
	2016	46	362	93.4	
	<b>2017</b>	<b>42</b>	<b>330</b>	<b>93.6</b>	

Table 4 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2017
North America	2001	4	32	87.5	Canada (9), United States of America (4)
	2002	2	16	100.0	
	2003	6	41	95.1	
	2004	8	55	81.8	
	2006	10	80	96.3	
	2007	12	94	97.9	
	2008	11	84	95.2	
	2009	12	90	92.2	
	2010	13	103	100.0	
	2011	11	81	97.6	
	2012	14	101	93.1	
	2013	13	92	97.8	
	2014	13	84	100.0	
	2015	13	93	100.0	
	2016	13	100	99.0	
	<b>2017</b>	<b>13</b>	<b>99</b>	<b>99.0</b>	
Oceania	2001	4	30	100.0	Australia (3), New Zealand
	2002	6	43	93.0	
	2003	6	46	93.5	
	2004	5	38	97.4	
	2006	5	37	94.6	
	2007	4	32	100.0	
	2008	4	30	93.3	
	2009	4	32	96.9	
	2010	4	32	100.0	
	2011	4	32	100.0	
	2012	4	32	100.0	
	2013	4	31	100.0	
	2014	4	32	100.0	
	2015	4	31	100.0	
	2016	4	32	100.0	
	<b>2017</b>	<b>4</b>	<b>31</b>	<b>100.0</b>	
Russia	2001	1	8	12.5	- none -
	2002	1	8	62.5	
	2003	1	7	14.3	
	2004	4	26	69.2	
	2006	5	40	80.0	
	2007	8	51	80.4	
	2008	6	40	90.0	
	2009	7	49	91.8	
	2010	8	54	87.1	
	2011	7	48	87.3	
	2012	6	48	87.5	
	2013	2	16	75.0	
	2014	4	30	93.3	
	2015	3	24	100.0	
	2016	-	-	-	
	<b>2017</b>	<b>-</b>	<b>-</b>	<b>-</b>	
Latin America	2001	11	78	57.7	Argentina, Bolivia, Brazil (2), Chile, Colombia (4), Costa Rica (2), Ecuador, Guatemala, Honduras, Mexico (3), Panama (2), Paraguay, Peru, Uruguay, Venezuela
	2002	11	82	87.8	
	2003	13	83	75.9	
	2004	15	88	79.5	
	2006	13	84	84.5	
	2007	15	107	88.8	
	2008	17	120	71.7	
	2009	21	150	77.3	
	2010	22	132	80.0	
	2011	23	144	83.7	
	2012	25	182	73.1	
	2013	22	154	83.1	
	2014	24	166	84.9	
	2015	20	133	84.2	
	2016	23	165	87.9	
	<b>2017</b>	<b>23</b>	<b>178</b>	<b>89.3</b>	



Table 4 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* serotyping

Region	EQAS iteration	No. of labs	No. of strains serotyped	% strains correctly serotyped	Countries participating in EQAS 2017
Southeast Asia	2001	15	113	54.0	Brunei Darussalam, Cambodia, Japan (2), Korea, Rep of (2), LAO PDR, Malaysia (4), Philippines (2), Singapore (2), Sri Lanka, Taiwan, Thailand (12), Viet Nam (2)
	2002	12	90	92.2	
	2003	15	100	81.0	
	2004	17	130	81.5	
	2006	15	117	84.6	
	2007	19	140	91.4	
	2008	18	125	81.6	
	2009	23	180	81.1	
	2010	24	172	90.5	
	2011	23	180	98.4	
	2012	28	207	77.8	
	2013	22	163	89.6	
	2014	22	166	94.6	
	2015	24	179	88.3	
	2016	28	211	87.7	
	<b>2017</b>	<b>31</b>	<b>244</b>	<b>89.3</b>	
China	2001	4	32	96.9	China (15)
	2002	3	24	100.0	
	2003	8	60	75.0	
	2004	7	46	78.3	
	2006	6	48	85.4	
	2007	10	80	91.3	
	2008	15	108	94.4	
	2009	16	126	95.2	
	2010	10	74	92.5	
	2012	10	78	80.8	
	2013	7	54	92.6	
	2014	9	71	93.0	
	2015	15	118	78.0	
	2016	17	136	95.6	
	<b>2017</b>	<b>15</b>	<b>120</b>	<b>97.5</b>	

Table 5. *Salmonella* serogroups (SG), serotypes (ST) and deviations (D), WHO EQAS 2017

Strain ID	Correct serotype		No. of labs reporting SG	% D <sub>SG</sub>	No. of labs reporting ST	% D <sub>ST</sub>	Deviating results (*)
WHO 2017 S-17.1	Infantis	I 6,7:r:1,5	159	4.4	143	11.2	Choleraesuis (2), Goma, Irumu, Papuana, Paratyphi A, Paratyphi C, Surat, Thompson, Typhi, Virchow (6)
WHO 2017 S-17.2	Havana	I 13,23:f,g;-	149	10.7	137	10.9	Adelaide, Derby, II .1.,13,23:g,m,[s],t:[e,n,x], Kiel, Linton, Lomita, Paratyphi A, Paratyphi C, Raus (3), Rideau (2), Rissen, Rissen var. 14+
WHO 2017 S-17.3	Enteritidis	I 9,12:g,m;-	156	1.3	142	2.1	Berta, Typhi (2)
WHO 2017 S-17.4	Rissen	I 6,7:f,g;-	152	1.3	139	10.1	Alamo, Eingedi (2), Galiema, Menston, Montevideo, Othmarschen (4), Plumaugat, Typhi
WHO 2017 S-17.5	Weltevreden	I 3,10:r;z6	155	2.6	142	9.2	Assinie, Dumfries, Elisabethville (3), Fareham, Paratyphi B (2), Simi, Stockholm, Ughelli (2)
WHO 2017 S-17.6	Schwarzengrund	I 4,12:d:1,7	156	1.3	141	10.6	Ahmadi, Ayinde, Brezany, Kisangani, Kubacha, Kaapstad, Paratyphi A, Paratyphi B, Sarajane, Stanley (2), Travis, Typhimurium (2), Uppsala
WHO 2017 S-17.7	Typhimurium	I 4,5,12:i;1,2	157	1.3	143	6.3	Avonmouth, I 1,4,5,12:i:, Lagos (3), Paratyphi A, Saintpaul
WHO 2017 S-17.8	Kentucky	I 8,20:i;z6	145	15.9	135	17.0	Azteca, Bardo, Bargny, Enteritidis, Falkensee, Haardt, Paratyphi A (2), Sekondi, Tumodi (14)

\*number of participants reporting the specified deviating result

Table 6. EQAS participating laboratories' performance of antimicrobial susceptibility testing of *Salmonella* strains

EQAS iteration	No. of EQAS participating laboratories	% correct test results	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (R → S & S → R)^	% total deviations (S → R & R → S & S ↔ I or I ↔ R)^
2000	44	92	4	4	0	4	8
2001	108	91	6	2	1	3	9
2002	119	92	6	2	1	3	9
2003*	147	93	4	3	0	3	7
2004	152	93	4	2	1	3	7
2006	143	88	8	3	1	4	12
2007	143	93	4	2	1	3	7
2008	168	91	4	2	3	5	9
2009	153	94	3	2	1	3	6
2010	152	92	4	3	2	5	8
2011	127	91	4	2	3	5	9
2012	159	94	3	2	1	3	6
2013	145	95	3	2	0	2	5
2014	155	95	3	1	1	2	5
2015	155	92	4	2	1	4	8
2016	150	95	2	2	1	3	5
2017	150	91	3	2	3	5	8
<b>Average*</b>	<b>139</b>	<b>93</b>	<b>4</b>	<b>2</b>	<b>1</b>	<b>4</b>	<b>8</b>

\*Data do not include one strain which may have lost resistance due to transport or storage stress

^S, susceptible; I, intermediate; R, resistant

Table 7. EQAS participants' performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial

EQAS iteration	No. of labs	Performance	Antimicrobial <sup>o</sup>																		OVERALL average
			AMC	AMP	CAZ	CHL	CIP	COL	CRO	CTX	GEN	KAN	NAL	SMX	MER	STR	SXT	TET	TMP	XNL	
2000	44	No. of tests	-	343	-	343	334	-	-	-	343	312	328	248	-	312	-	335	295	-	798
		% critical deviations*	-	6	-	4	1	-	-	-	4	4	1	3	-	4	-	6	1	-	6
		% total deviations^	-	8	-	7	6	-	-	-	5	16	4	5	-	12	-	13	1	-	14
2001	108	No. of tests	-	822	-	814	813	-	-	-	821	623	726	431	-	679	757	804	416	-	1778
		% critical deviations*	-	4	-	2	1	-	-	-	2	2	2	6	-	7	2	7	1	-	6
		% total deviations^	-	7	-	3	4	-	-	-	4	7	8	9	-	27	5	18	2	-	15
2002	119	No. of tests	-	918	-	903	911	-	-	-	905	680	885	495	-	718	724	861	499	-	1961
		% critical deviations*	-	2	-	2	0	-	-	-	2	2	2	4	-	4	7	3	3	-	5
		% total deviations^	-	3	-	3	2	-	-	-	16	10	4	4	-	34	10	7	3	-	15
2003*	147	No. of tests	-	1019	-	996	995	-	-	-	993	738	947	615	-	768	929	995	582	-	2210
		% critical deviations*	-	2	-	1	0	-	-	-	2	2	1	4	-	9	2	4	1	-	5
		% total deviations^	-	4	-	2	1	-	-	-	2	6	4	5	-	39	2	11	1	-	12
2004	152	No. of tests	973	1178	-	1159	1162	-	-	995	1201	-	1130	734	-	947	1051	1122	729	-	2653
		% critical deviations*	6	3	-	2	0	-	-	0	2	-	1	5	-	1	3	5	2	-	5
		% total deviations^	12	5	-	2	1	-	-	14	3	-	4	8	-	21	4	11	2	-	13
2006	143	No. of tests	950	1092	769	1060	1110	-	-	956	1078	-	1035	649	-	896	996	1054	607	225	2256
		% critical deviations*	9	2	7	3	2	-	-	7	3	-	2	6	-	5	3	9	1	2	8
		% total deviations^	22	3	11	15	6	-	-	15	7	-	6	7	-	22	5	20	2	9	21
2007	143	No. of tests	908	1114	830	1105	1101	-	-	914	1111	-	1092	678	-	875	971	1047	583	258	2290
		% critical deviations*	6	5	1	0	1	-	-	1	3	-	2	5	-	4	3	4	1	0	5
		% total deviations^	17	7	1	6	1	-	-	2	4	-	3	6	-	26	3	11	2	6	13
2008	168	No. of tests	-	1331	961	1226	1307	-	791	1104	1265	-	1168	718	-	867	1155	1249	696	-	2769
		% critical deviations*	-	3	3	1	19	-	3	3	4	-	2	4	-	7	3	6	2	-	8
		% total deviations^	-	8	6	11	21	-	6	6	6	-	4	5	-	25	4	13	2	-	16
2009	153	No. of tests	-	1206	921	1108	1190	-	775	1009	1143	-	1095	624	-	864	1042	1114	616	-	2541
		% critical deviations*	-	3	1	1	8	-	0	1	2	-	1	7	-	9	3	4	1	-	6
		% total deviations^	-	6	1	2	10	-	1	2	3	-	3	9	-	30	4	10	1	-	11
2010	152	No. of tests	-	1173	937	1118	1194	-	787	1026	1133	-	1096	566	-	800	1012	1134	604	-	2516
		% critical deviations*	-	4	2	1	3	-	4	4	5	-	1	14	-	19	4	5	1	-	9
		% total deviations^	-	5	3	2	3	-	8	8	6	-	2	17	-	55	4	9	1	-	17

Table 7 (continued). EQAS participants' performance of *Salmonella* strains antimicrobial susceptibility testing categorized by antimicrobial.

EQAS iteration	No. of labs	Performance	Antimicrobial <sup>°</sup>																		OVERALL Average
			AMC	AMP	CAZ	CHL	CIP	COL	CRO	CTX	GEN	KAN	NAL	SMX	MER	STR	SXT	TET	TMP	XNL	
2011	127	No. of tests	-	1099	829	988	1070	-	744	909	999	-	993	542	-	682	988	1017	493	-	2271
		% critical deviations*	-	5	3	2	20	-	3	4	4	-	7	4	-	3	3	4	1	-	9
		% total deviations^	-	6	4	2	21	-	3	6	5	-	15	5	-	42	3	10	2	-	17
2012	159	No. of tests	-	1228	993	1159	1245	-	834	1058	1161	-	1136	584	-	814	1054	1163	613	-	2608
		% critical deviations*	-	3	2	1	11	-	2	4	3	-	2	5	-	2	1	2	1	-	5
		% total deviations^	-	5	2	2	12	-	3	5	4	-	4	7	-	35	2	5	1	-	12
2013	145	No. of tests	-	1121	898	1027	1134	-	763	1011	1086	-	1027	491	-	-	946	1060	545	-	2381
		% critical deviations*	-	2	3	0	2	-	1	3	3	-	2	4	-	-	2	3	2	-	4
		% total deviations^	-	3	3	1	18	-	2	6	6	-	6	5	-	-	2	5	2	-	9
2014	155	No. of tests	-	1176	1003	1072	1161	-	817	1014	1147	-	1078	561	-	-	1039	1107	541	-	2511
		% critical deviations*	-	3	3	1	3	-	1	2	3	-	1	5	-	-	2	3	2	-	4
		% total deviations^	-	4	4	2	19	-	2	3	5	-	2	6	-	-	3	5	2	-	9
2015	155	No. of tests	-	1176	1010	1064	1172	-	787	1018	1145	-	1010	514	611	-	1034	1077	591	-	2468
		% critical deviations*	-	3	9	2	1	-	3	5	3	-	4	7	1	-	2	2	2	-	6
		% total deviations^	-	5	11	22	14	-	4	6	5	-	10	9	1	-	3	5	2	-	13
2016	150	No. of tests	-	1133	988	1020	1100	-	800	968	1104	-	959	529	838	-	953	1042	599	-	2407
		% critical deviations*	-	4	4	1	1	-	2	4	4	-	1	7	5	-	2	3	2	-	8
		% total deviations^	-	5	4	2	10	-	3	4	6	-	3	8	6	-	2	6	2	-	12
2017	150	No. of tests	-	1166	1016	881	1167	473	831	968	1113	-	921	487	921	-	1055	1014	553	-	1354
		% critical deviations*	-	4	17	4	1	6	6	6	4	-	2	5	6	-	6	2	5	-	3
		% total deviations^	-	5	22	5	18	6	7	8	5	-	2	7	6	-	7	4	5	-	9
Average*	139	No. of tests	944	1076	930	1003	1069	473	793	996	1044	588	978	557	790	769	982	1011	562	242	800
		% critical deviations*	7	3	5	2	4	6	3	3	3	3	2	6	4	6	3	4	2	1	4
		% total deviations^	17	5	6	4	10	6	4	7	5	10	5	7	4	31	4	10	2	8	8

<sup>°</sup>For antimicrobial abbreviations: see List of Abbreviations page 1

\*R→ S & S → R (R, resistant; S, susceptible)

^S→R & R→S & S↔I or I↔R (I, intermediate)

• Data do not include one strain which may have lost resistance due to transport or storage stress

-, not determined

Table 8. Antimicrobial susceptibility test results (number of R/I/S) for the EQAS 2017 *Salmonella* strains\*

Strain	Antimicrobial <sup>^</sup>														
	AMP	CTX	FOX	CAZ	CRO	CHL	CIP	COL	GEN	MER	NAL	SMX	TET	TMP	SXT
WHO S-17.1	132/0/13	101/2/18	9/4/101	77/15/34	90/0/15	5/2/120	15/73/56	2/0/58	6/1/131	9/1/106	111/1/1	60/0/2	124/2/1	50/0/19	88/1/38
WHO S-17.2	10/2/134	16/8/96	31/36/46	53/14/60	7/0/97	1/0/124	4/11/132	1/0/58	4/2/133	5/0/109	3/2/111	14/7/40	2/2/123	1/0/68	5/1/126
WHO S-17.3	9/6/130	7/3/112	-	5/6/117	8/1/95	*	1/17/127	5/1/53	124/5/7	4/0/109	2/1/113	56/1/5	11/9/106	2/0/69	5/0/127
WHO S-17.4	142/1/3	117/2/2	7/1/107	55/18/54	97/1/6	115/0/10	0/15/130	1/1/57	5/2/132	4/0/111	0/1/114	56/0/4	122/2/3	64/0/5	122/0/11
WHO S-17.5	6/0/140	3/2/116	-	5/0/123	5/1/97	122/2/1	1/13/132	2/1/55	5/0/135	5/0/110	3/1/112	60/0/0	125/1/1	69/0/0	132/1/0
WHO S-17.6	6/0/140	2/3/116	-	4/1/122	2/0/101	7/0/119	0/15/129	4/0/54	4/1/135	4/0/110	3/1/110	60/0/0	124/2/1	69/0/0	128/0/5
WHO S-17.7	141/0/5	10/2/109	-	6/4/117	7/0/96	119/1/6	30/92/26	52/0/10	4/1/135	8/0/107	112/1/3	60/0/0	124/2/1	69/0/0	131/0/1
WHO S-17.8	146/0/0	120/1/0	111/2/0	125/1/0	101/0/4	121/0/3	140/4/4	2/0/56	10/1/130	101/6/12	115/0/0	62/0/0	122/2/2	68/0/0	131/0/2

<sup>^</sup>For antimicrobial abbreviations: see List of Abbreviations page 1

Marked in bold: expected interpretation. Grey cell: <90% of laboratories did correct interpretation. R, resistant/I, intermediate/ S, susceptible.

\*The result for the *Salmonella* strain WHO S-17.3 for chloramphenicol was omitted from evaluation (during the process of analyzing the WHO EQAS 2017 data, it was clear to the organizers that the database evaluation of the result related to the *Salmonella* strain WHO S-17.3 for chloramphenicol caused a large number of deviations. The expected result related to the testing of WHO S-17.3/chloramphenicol was 16/intermediate, only, due to the large number of deviations, the organizers decided not to evaluate the submitted results related to this strain/antimicrobial combination.)

Table 9. Region-based categorization of EQAS participants' performance of *Salmonella* AST

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
Africa	2001	7	80.1	9.6	7.7	2.5	10.2	19.8	Cameroun, Egypt, Ghana, Kenya (2), Madagascar, Mauritius, Morocco, Nigeria (4), South Africa, Tanzania, United Republic of, The Gambia, Zambia, Zimbabwe
	2002	10	94.3	4.1	1.0	0.6	1.6	5.7	
	2003	13	86.9	6.6	2.8	3.7	6.5	13.1	
	2004	11	85.7	7.2	5.2	1.9	7.1	14.3	
	2006	20	85.8	7.5	4.1	2.7	6.8	14.3	
	2007	16	90.7	4.4	4.0	0.9	4.9	9.3	
	2008	19	83.8	6.5	5.5	4.2	9.7	16.2	
	2009	22	90.1	4.5	3.6	1.8	5.4	9.9	
	2010	22	84.7	6.0	6.5	2.8	9.3	15.3	
	2011	17	87.0	5.0	4.7	3.3	8.0	13.0	
	2012	18	89.4	5.3	3.5	1.9	5.4	10.6	
	2013	16	92.0	3.2	4.0	0.9	4.9	8.0	
	2014	20	92.5	3.8	2.0	1.7	3.7	7.5	
	2015	22	86.7	7.3	4.1	1.9	6.0	13.3	
	2016	18	90.1	4.6	4.2	1.1	5.3	9.9	
	<b>2017</b>	<b>17</b>	<b>87.2</b>	<b>4.5</b>	<b>4.0</b>	<b>4.3</b>	<b>8.3</b>	<b>12.8</b>	
Central Asia & Middle East	2001	10	87.7	6.3	5.2	0.8	6.0	12.3	Bangladesh, India (4), Iran, Islamic rep. of (3), Israel, Nepal (6)
	2002	6	83.4	9.8	6.6	0.2	6.8	16.6	
	2003	8	89.9	4.5	4.0	1.6	5.6	10.1	
	2004	10	87.5	6.7	5.5	0.3	5.8	12.5	
	2006	7	79.2	10.5	9.8	0.5	10.3	20.8	
	2007	8	87.8	5.0	6.2	1.1	7.3	12.2	
	2008	12	86.1	6.5	4.0	3.4	7.4	13.9	
	2009	6	93.7	4.3	0.9	1.1	2.0	6.3	
	2010	7	95.8	2.6	0.2	1.4	1.6	4.2	
	2011	4	91.8	4.1	1.8	2.3	4.1	8.2	
	2012	8	92.8	4.4	1.6	0.7	2.3	6.6	
	2013	8	93.6	5.2	1.0	0.1	1.2	6.4	
	2014	17	91.0	4.2	2.9	2.0	4.9	9.0	
	2015	14	91.4	4.3	2.3	2.1	4.4	8.6	
	2016	11	95.5	0.9	1.8	1.8	3.6	4.5	
	<b>2017</b>	<b>15</b>	<b>88.9</b>	<b>5.0</b>	<b>2.6</b>	<b>3.5</b>	<b>6.1</b>	<b>11.1</b>	
Caribbean	2001	2	83.5	9.5	7.0	0.0	7.0	16.5	Barbados, Cuba, Curaçao, Jamaica, Trinidad and Tobago
	2002	1	95.8	4.2	0.0	0.0	0.0	4.2	
	2003	8	91.7	6.4	1.5	0.5	2.0	8.4	
	2004	8	94.1	3.1	1.9	0.9	2.8	5.9	
	2006	5	92.1	5.4	1.6	1.0	2.6	8.0	
	2007	4	95.0	3.1	0.9	0.9	1.8	5.0	
	2008	5	90.7	5.5	0.9	2.9	3.8	9.3	
	2009	4	93.2	1.8	3.2	1.8	5.0	6.8	
	2010	4	90.9	5.4	2.7	0.7	3.4	8.8	
	2011	2	96.5	1.4	0.0	2.1	2.1	3.5	
	2012	4	91.1	1.5	6.7	0.7	7.4	8.9	
	2013	3	90.2	2.6	7.3	0.0	7.3	9.8	
	2014	4	78.3	4.7	9.4	7.6	17.0	21.7	
	2015	4	87.5	6.6	3.7	2.2	5.9	12.5	
	2016	2	100.0	0.0	0.0	0.0	0.0	0.0	
	<b>2017</b>	<b>5</b>	<b>75.7</b>	<b>5.0</b>	<b>10.1</b>	<b>9.1</b>	<b>19.2</b>	<b>24.3</b>	

Table 9 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* antimicrobial susceptibility testing

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
Europe	8,5	47	91.3	5.7	2.7	0.3	3.0	8.7	Belgium, Croatia, Cyprus, Czech Republic, Denmark, Greece (3), Ireland, Italy (8), Kosova, Luxembourg (2), Malta, Poland (3), Portugal, Serbia (2), Slovak Republic, Slovenia, Spain, Turkey, Ukraine, United Kingdom
	2002	57	92.7	5.2	1.2	0.9	2.1	7.3	
	2003	64	92.9	3.8	1.0	2.3	3.3	7.1	
	2004	58	93.5	4.3	1.4	0.8	2.2	6.5	
	2006	54	88.7	7.0	3.8	0.6	4.4	11.3	
	2007	49	94.2	3.7	1.6	0.4	2.0	5.7	
	2008	51	91.2	4.4	2.5	1.9	4.4	8.8	
	2009	40	95.1	2.6	1.3	0.9	2.2	4.8	
	2010	39	92.4	4.1	1.2	2.3	3.5	7.6	
	2011	36	92.5	4.5	1.7	1.3	3.0	7.5	
	2012	40	95.5	2.8	1.2	0.4	1.7	4.5	
	2013	37	95.7	2.5	1.4	0.3	1.7	4.2	
	2014	40	96.6	2.1	0.8	0.5	1.3	3.4	
	2015	38	93.4	4.1	1.3	1.2	2.5	6.6	
	2016	36	96.9	1.5	1.2	0.5	1.6	3.1	
	<b>2017</b>	<b>33</b>	<b>92.8</b>	<b>2.4</b>	<b>2.1</b>	<b>2.7</b>	<b>4.8</b>	<b>7.2</b>	
North America	2001	4	95.8	3.8	0.0	0.4	0.4	4.2	Canada (5), United States of America (4)
	2002	3	90.5	6.9	0.6	2.0	2.6	9.5	
	2003	7	93.4	5.2	0.0	1.4	1.4	6.6	
	2004	9	94.2	4.2	1.8	0.0	1.8	6.0	
	2006	8	94.8	2.9	1.0	1.3	2.3	5.2	
	2007	10	95.4	2.9	0.8	0.8	1.6	4.6	
	2008	14	96.4	0.6	0.4	2.6	3.0	3.6	
	2009	10	98.7	0.0	0.4	0.9	1.3	1.3	
	2010	11	94.8	2.6	0.2	2.4	2.6	5.2	
	2011	9	92.1	2.6	1.5	3.8	5.3	7.9	
	2012	10	96.0	2.1	1.0	0.9	1.9	4.0	
	2013	7	98.4	1.3	0.0	0.2	0.2	1.6	
	2014	8	96.9	2.2	0.4	0.6	0.9	3.1	
	2015	8	94.5	2.0	0.8	2.8	3.6	5.5	
	2016	8	99.1	0.2	0.0	0.7	0.7	0.9	
	<b>2017</b>	<b>9</b>	<b>97.1</b>	<b>1.2</b>	<b>0.6</b>	<b>1.2</b>	<b>1.7</b>	<b>2.9</b>	
Oceania	2001	6	91.8	4.7	2.7	0.9	3.6	8.2	Australia, New Zealand
	2002	7	91.7	6.2	0.0	2.0	2.0	8.3	
	2003	9	94.3	2.5	1.2	2.0	3.2	5.7	
	2004	11	97.1	2.5	0.3	0.1	0.4	2.9	
	2006	7	93.4	4.6	0.9	1.1	2.0	6.6	
	2007	1	98.9	1.1	0.0	0.0	0.0	1.1	
	2008	4	93.9	3.8	0.0	2.3	2.3	6.1	
	2009	4	95.9	3.2	0.3	0.6	0.9	4.1	
	2010	4	92.5	4.6	0.6	2.3	2.9	7.5	
	2011	4	93.8	5.6	0.6	0.0	0.6	6.2	
	2012	4	95.5	3.1	0.6	0.9	1.4	4.5	
	2013	4	96.8	2.9	0.0	0.3	0.3	3.2	
	2014	5	97.4	2.0	0.0	0.6	0.6	2.6	
	2015	5	95.3	3.8	0.5	0.5	1.0	4.8	
	2016	3	98.1	0.0	0.5	1.4	1.9	1.9	
	<b>2017</b>	<b>2</b>	<b>96.1</b>	<b>2.6</b>	<b>0.0</b>	<b>1.3</b>	<b>1.3</b>	<b>3.9</b>	



Table 9 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* antimicrobial susceptibility testing.

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
Russia	2001	1	81.9	15.3	2.8	0.0	2.8	18.1	- none -
	2002	1	84.5	9.9	5.6	0.0	5.6	15.5	
	2003	1	100.0	0.0	0.0	0.0	0.0	0.0	
	2004	4	91.2	6.6	1.5	0.7	2.2	8.8	
	2006	5	87.4	8.2	2.7	1.7	4.4	12.6	
	2007	8	88.9	5.8	4.8	0.4	5.2	11.0	
	2008	6	92.2	4.7	1.4	1.7	3.1	7.8	
	2009	6	93.8	2.1	3.3	0.8	4.1	6.2	
	2010	8	94.3	3.3	1.3	1.1	2.4	5.7	
	2011	7	90.0	4.8	3.2	2.0	5.2	10.0	
	2012	6	97.4	2.0	0.0	0.6	0.6	2.6	
	2013	2	98.2	1.8	0.0	0.0	0.0	1.8	
	2014	4	98.2	0.3	0.9	0.6	1.5	1.8	
	2015	4	98.7	1.0	0.0	0.3	0.3	1.3	
	2016	-	-	-	-	-	-	-	
	<b>2017</b>	-	-	-	-	-	-	-	
Latin America	2001	11	90.8	6.9	1.4	1.0	2.4	9.2	Argentina, Belize, Bolivia, Brazil (2), Chile, Colombia (3), Costa Rica (2), Ecuador, Guatemala (2), Honduras, Mexico (2), Panama (2), Paraguay, Peru, Suriname, Uruguay, Venezuela
	2002	13	93.7	4.6	0.7	1.0	1.7	6.3	
	2003	12	90.8	4.2	2.0	3.0	5.0	9.2	
	2004	17	94.4	4.7	0.8	0.1	0.9	5.6	
	2006	16	88.7	6.3	4.5	0.6	5.1	11.3	
	2007	17	94.9	1.8	1.9	1.4	3.3	5.0	
	2008	20	93.0	3.4	1.5	2.1	3.6	7.0	
	2009	20	95.6	2.1	1.1	1.2	2.3	4.4	
	2010	23	90.8	2.1	5.6	1.4	7.1	9.2	
	2011	22	90.8	2.8	3.1	3.3	6.4	9.2	
	2012	25	94.4	1.6	3.0	1.0	4.0	5.6	
	2013	25	95.5	2.6	1.2	0.3	1.5	4.2	
	2014	24	96.5	1.9	1.1	0.6	1.7	3.5	
	2015	20	94.9	3.8	0.6	0.7	1.3	5.1	
	2016	24	95.6	2.5	1.4	0.5	1.9	4.4	
	<b>2017</b>	<b>24</b>	<b>91.1</b>	<b>3.3</b>	<b>2.3</b>	<b>3.2</b>	<b>5.5</b>	<b>8.9</b>	
China	2001	4	98.9	0.8	0.0	0.3	0.3	1.1	China (14)
	2002	3	96.0	4.0	0.0	0.0	0.0	4.0	
	2003	8	90.1	3.6	2.8	3.6	6.4	10.0	
	2004	8	96.0	3.2	0.7	0.1	0.8	4.0	
	2006	6	89.6	7.0	2.9	0.5	3.4	10.4	
	2007	10	98.3	1.1	0.3	0.2	0.5	1.6	
	2008	18	92.8	3.7	0.8	2.7	3.5	7.2	
	2009	14	94.8	2.2	2.1	0.8	2.9	5.1	
	2010	9	92.1	4.5	1.6	1.8	3.4	7.9	
	2012	9	95.3	3.0	0.5	1.2	1.6	4.7	
	2013	8	96.9	2.0	0.5	0.5	1.0	3.1	
	2014	8	97.0	1.2	0.1	1.6	1.8	3.0	
	2015	15	92.8	2.0	4.0	1.1	5.1	7.2	
	2016	16	96.7	0.4	1.8	1.1	2.9	3.3	
	<b>2017</b>	<b>14</b>	<b>93.4</b>	<b>2.9</b>	<b>0.7</b>	<b>3.0</b>	<b>3.7</b>	<b>6.6</b>	

^S. susceptible; I. intermediate; R. resistant

Table 9 (continued). Region-based categorization of EQAS participants' performance of *Salmonella* antimicrobial susceptibility testing.

Region	EQAS iteration	No. of labs	% correct test result	% minor deviations (S ↔ I or I ↔ R)^	% major deviations (S → R)^	% very major deviations (R → S)^	% critical deviations (S → R & R → S)^	% total deviations (S→R & R→S & S↔I or I↔R)^	Countries participating in the 2017 iteration
Southeast Asia	2001	16	88.1	7.7	2.3	1.9	4.2	11.9	Cambodia, Japan (2), Korea, Rep of (2), LAO PDR, Malaysia (5), Philippines (2), Singapore, Sri Lanka (2), Taiwan, Thailand (13), Viet Nam
	2002	18	89.0	8.1	1.4	1.6	3.0	11.0	
	2003	17	87.4	5.2	4.7	2.7	7.4	12.6	
	2004	16	92.8	4.4	2.3	0.5	2.8	7.2	
	2006	15	90.0	8.1	1.2	0.8	2.0	10.0	
	2007	20	93.9	4.0	1.4	0.7	2.1	6.1	
	2008	19	90.5	4.7	2.2	2.6	4.8	9.5	
	2009	27	91.8	4.1	3.0	1.2	4.2	8.3	
	2010	25	92.8	3.8	1.5	1.9	3.4	7.2	
	2011	26	90.5	3.5	2.4	3.5	5.9	9.5	
	2012	35	91.7	3.9	3.5	0.9	4.4	8.3	
	2013	35	93.4	3.2	2.5	0.7	3.2	6.4	
	2014	8	97.0	1.2	0.1	1.6	1.8	3.0	
	2015	25	89.9	6.0	2.6	1.5	4.1	10.1	
	2016	30	93.5	2.2	3.5	0.8	4.3	6.5	
	<b>2017</b>	<b>31</b>	<b>91.9</b>	<b>2.9</b>	<b>2.1</b>	<b>3.2</b>	<b>5.2</b>	<b>8.1</b>	

^S. susceptible; I. intermediate; R. resistant

Table 10. EQAS participants' performance of antimicrobial susceptibility testing of quality control strain *Escherichia coli* ATCC 25922

		Method	Performance <sup>4,5</sup>	AMP	CAZ	CHL	CIP	COL	CRO	CTX	FIS (SMX) <sup>2</sup>	FOX	GEN	MER	NAL	STR	SXT	TET	TMP
Accepted interval <sup>1</sup>		MIC (µg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.25-2	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 <sup>3</sup>	≤0.5/9.5	0.5-2	0.5-2
		Disks (mm)		15-22	25-32	21-27	30-40	-	29-35	29-35	15-23	23-29	19-26	28-34	22-28	12-20	23-29	18-25	21-28
EQAS iteration (total no. of participants)	2000 (44)	MIC & Disk	No. <sup>4</sup>	37	-	38	35	-	-	-	19	-	39	-	37	36	-	42	31
			% <sup>5</sup>	27	-	37	20	-	-	-	53	-	23	-	35	22	-	42	30
	2001 (107)	MIC & Disk	No. <sup>4</sup>	97	-	97	97	-	-	-	53	-	99	-	74	81	90	96	50
			% <sup>5</sup>	19	-	20	14	-	-	-	34	-	12	-	14	12	14	22	22
	2002 (114)	MIC & Disk	No. <sup>4</sup>	109	-	107	108	-	-	-	57	-	108	-	102	82	102	102	66
			% <sup>5</sup>	16	-	15	14	-	-	-	26	-	12	-	14	11	12	13	11
	2003 (144)	MIC & Disk	No. <sup>4</sup>	140	-	137	138	-	-	-	82	-	138	-	132	105	129	137	79
			% <sup>5</sup>	14	-	22	9	-	-	-	17	-	9	-	16	9	14	19	14
	2004 (140)	MIC & Disk	No. <sup>4</sup>	132	-	128	132	-	-	111	84	-	134	-	126	110	120	129	87
			% <sup>5</sup>	10	-	13	8	-	-	18	16	-	10	-	9	6	11	13	9
	2006 (137)	MIC & Disk	No. <sup>4</sup>	133	96	126	127	-	-	115	74	-	131	-	122	106	122	125	74
			% <sup>5</sup>	14	15	18	8	-	-	21	29	-	14	-	20	11	19	12	17
	2007 (126)	MIC & Disk	No. <sup>4</sup>	124	92	123	121	-	-	104	64	-	124	-	120	97	107	117	67
			% <sup>5</sup>	11	9	14	12	-	-	16	22	-	6	-	7	6	13	7	10
	2008 (147)	MIC & Disk	No. <sup>4</sup>	147	111	135	144	-	-	124	71	-	145	-	136	101	129	139	79
			% <sup>5</sup>	12	9	10	8	-	-	14	14	-	8	-	8	12	13	7	13
		MIC	No. <sup>4</sup>	33	23	24	33	-	-	23	18	-	31	-	23	19	22	28	16
			% <sup>5</sup>	0	5	0	6	-	-	9	11	-	0	-	0	11	9	0	13
			No. <sup>4</sup>	114	89	112	111	-	-	101	53	-	114	-	113	82	107	111	63
			% <sup>5</sup>	16	10	12	8	-	-	15	15	-	11	-	10	12	14	9	13
	2009 (129)	MIC & Disk	No. <sup>4</sup>	128	100	121	124	-	88	107	63	-	123	-	117	98	113	122	70
			% <sup>5</sup>	16	13	15	7	-	16	10	11	-	18	-	13	10	14	14	11
		MIC (27)	No. <sup>4</sup>	27	19	24	26	-	20	20	14	-	25	-	24	19	21	27	25
			% <sup>5</sup>	11	11	8	8	-	15	15	21	-	12	-	8	5	19	11	13
		Disk (102)	No. <sup>4</sup>	101	81	97	98	-	68	87	49	-	98	-	93	79	92	95	55
			% <sup>5</sup>	16	14	16	6	-	16	9	10	-	18	-	14	11	12	15	11
	2010 (116)	MIC & Disk	No. <sup>4</sup>	114	97	108	115	-	79	100	51	-	112	-	104	84	101	110	63
			% <sup>5</sup>	11	9	9	6	-	10	14	11	-	11	-	5	5	12	5	15
		MIC (24)	No. <sup>4</sup>	25	15	21	25	-	15	17	12	-	24	-	19	17	17	24	11
			% <sup>5</sup>	12	20	10	8	-	7	18	8	-	13	-	16	18	18	17	36
		Disk (91)	No. <sup>4</sup>	89	82	87	90	-	64	83	39	-	88	-	85	67	84	86	52
			% <sup>5</sup>	9	6	8	4	-	9	11	10	-	9	-	2	1	10	1	8
	2011 (112)	MIC & Disk	No. <sup>4</sup>	111	89	102	109	-	76	96	50	-	103	-	103	72	99	107	51
			% <sup>5</sup>	17	4	11	7	-	7	9	8	-	11	-	8	4	16	7	14
		MIC (23)	No. <sup>4</sup>	23	15	18	22	-	16	15	13	-	22	-	19	17	16	21	11
			% <sup>5</sup>	4	7	0	9	-	6	0	8	-	9	-	0	6	6	5	0
		Disk (89)	No. <sup>4</sup>	88	74	84	87	-	60	81	37	-	81	-	84	55	83	86	40
			% <sup>5</sup>	20	4	13	7	-	7	11	8	-	11	-	10	4	18	8	18

Table 10 (continued). EQAS participants' performance of antimicrobial susceptibility testing of quality control strain *Escherichia coli* ATCC 25922

		Method	Performance <sup>4,5</sup>	AMP	CAZ	CHL	CIP	COL	CRO	CTX	FIS (SMX) <sup>2</sup>	FOX	GEN	MER	NAL	STR	SXT	TET	TMP
Accepted interval <sup>1</sup>		MIC (µg/ml)		2-8	0.06-0.5	2-8	0.004-0.016	0.25-2	0.03-0.12	0.03-0.12	8-32	2-8	0.25-1	0.008-0.06	1-4	4-16 <sup>3</sup>	≤0.5/9.5	0.5-2	0.5-2
		Disks (mm)		15-22	25-32	21-27	30-40	-	29-35	29-35	15-23	23-29	19-26	28-34	22-28	12-20	23-29	18-25	21-28
EQAS iteration (total no. of participants)	2012 (135)	MIC & Disk	No. <sup>4</sup>	134	111	121	131	-	90	115	53	-	127	-	121	89	112	129	66
			% <sup>5</sup>	13	12	7	6	-	11	10	11	-	9	-	9	8	13	10	21
		MIC (37)	No. <sup>4</sup>	37	26	31	35	-	23	28	19	-	35	-	31	26	23	35	22
			% <sup>5</sup>	3	4	0	3	-	0	4	5	-	3	-	3	8	0	0	9
	Disk (98)	No. <sup>4</sup>	97	85	90	96	-	67	87	34	-	92	-	90	63	89	94	44	
		% <sup>5</sup>	16	14	9	7	-	15	11	15	-	11	-	11	8	16	14	27	
	2013 (122)	MIC & Disk	No. <sup>4</sup>	117	100	112	119	-	82	107	44	-	113	-	113	-	101	114	59
			% <sup>5</sup>	12	7	5	7	-	4	8	10	-	6	-	11	-	8	8	11
		MIC (33)	No. <sup>4</sup>	31	25	28	32	-	19	27	17	-	32	-	28	-	22	32	22
			% <sup>5</sup>	6	4	4	13	-	5	11	18	-	9	-	11	-	5	6	5
		Disk (89)	No. <sup>4</sup>	86	75	84	87	-	63	80	27	-	81	-	85	-	79	82	37
			% <sup>5</sup>	13	8	6	5	-	5	6	7	-	4	-	9	-	10	7	8
	2014 (115)	MIC & Disk	No. <sup>4</sup>	111	99	101	108	-	75	97	49	-	111	-	103	-	102	104	50
			% <sup>5</sup>	5	7	7	6	-	7	14	14	-	8	-	8	-	8	7	2
		MIC (28)	No. <sup>4</sup>	27	21	24	27	-	16	22	16	-	28	-	24	-	21	25	12
			% <sup>5</sup>	4	5	4	15	-	6	14	0	-	14	-	8	-	14	0	0
		Disk (87)	No. <sup>4</sup>	84	78	77	81	-	59	75	33	-	83	-	79	-	81	79	38
			% <sup>5</sup>	6	8	8	4	-	7	15	21	-	6	-	8	-	6	9	3
	2015 (117)	MIC&Disk	No. <sup>4</sup>	113	101	101	112	-	78	99	54	75	112	74	100	-	104	106	57
			% <sup>5</sup>	8	5	7	7	-	9	6	11	9	9	12	7	-	13	8	9
		MIC (31)	No. <sup>4</sup>	30	26	25	30	-	16	25	15	20	30	19	24	-	24	27	16
			% <sup>5</sup>	3	8	4	13	-	0	12	7	10	7	11	4	-	8	7	13
		Disk (85)	No. <sup>4</sup>	83	75	76	82	-	62	74	39	55	82	55	76	-	80	79	41
			% <sup>5</sup>	10	4	8	5	-	11	4	13	9	10	13	8	-	14	8	7
	2016 (106)	MIC&Disk	No. <sup>4</sup>	101	93	95	101	-	76	94	54	84	99	88	91	-	91	97	59
			% <sup>5</sup>	11	5	13	9	-	16	15	24	7	8	10	9	-	8	10	14
		MIC (30)	No. <sup>4</sup>	27	24	24	27	-	17	24	13	22	29	25	20	-	20	25	16
			% <sup>5</sup>	4	4	0	7	-	12	4	23	0	3	4	0	-	0	8	13
		Disk (76)	No. <sup>4</sup>	74	69	71	74	-	59	70	41	62	70	63	71	-	71	72	43
			% <sup>5</sup>	14	6	17	9	-	17	19	24	10	10	13	11	-	10	11	14
	2017 (115)	MIC&Disk	No. <sup>4</sup>	114	101	103	113	56	82	93	56	92	107	93	89	-	95	99	61
			% <sup>5</sup>	13	11	10	10	20	16	14	27	10	7	10	7	-	6	10	8
		MIC (41)	No. <sup>4</sup>	41	33	35	41	28	25	31	24	30	38	34	31	-	29	34	26
			% <sup>5</sup>	5	6	0	7	4	12	6	17	3	5	6	3	-	0	6	0
		Disk (74)	No. <sup>4</sup>	73	68	68	72	28	57	62	32	62	69	59	58	-	66	65	35
			% <sup>5</sup>	18	13	15	11	36	18	18	34	13	7	12	9	-	9	12	14

<sup>0</sup>For antimicrobial abbreviations: see List of Abbreviations page 1<sup>1</sup>CLSI standard. Performance Standards for Antimicrobial Disk and Dilution Susceptibility testing. 22nd Informational supplement. CLSI document M100-S22. 2012 Wayne. PA. USA<sup>2</sup>FIS (sulfisoxazole) covers the group of SMX (sulfonamides); <sup>3</sup>Quality control range developed by the manufacturer of Sensititre®; <sup>4</sup>No.. number of laboratories performing the analysis; <sup>5</sup>%. percentage of laboratories reporting erroneous results; -, not determined

Table 11. Proportion of laboratories that obtained the expected result. Number (n/N) and percentages of laboratories which correctly detected and confirmed the ESBL-producing *Salmonella* strains.

Isolate no.	Expected interpretation	Confirmatory tests
<b>WHO 2017 S-17.1</b>	Presumptive ESBL-phenotype	80/92 (87%)
<b>WHO 2017 S-17.2</b>	Presumptive AmpC-phenotype	26/77 (34%)
<b>WHO 2017 S-17.3</b>	-	-
<b>WHO 2017 S-17.4</b>	Presumptive ESBL-phenotype	82/91 (90%)
<b>WHO 2017 S-17.5</b>	-	-
<b>WHO 2017 S-17.6</b>	-	-
<b>WHO 2017 S-17.7</b>	-	-
<b>WHO 2017 S-17.8</b>	Presumptive carbapenemase-phenotype	62/94 (66%)

G00-06-001/01.12.2014

Kgs. Lyngby, Denmark, May 2017

## **SIGN-UP FOR EQAS 2017**

Greetings to the WHO Global Foodborne Infections Network (WHO GFN) Members:

WHO GFN strives to increase the quality of laboratory-based surveillance of *Salmonella* by encouraging national and regional reference laboratories that attended WHO GFN training courses to participate in the External Quality Assurance System (EQAS). We are pleased to announce the launch of the 2017 EQAS cycle.

### **WHY PARTICIPATE IN EQAS?**

EQAS provides the opportunity for proficiency testing which is considered an important tool for the production of reliable laboratory results of consistently good quality.

### **WHAT IS OFFERED IN EQAS?**

This year, WHO EQAS offers the following components:

- Serogrouping, serotyping and antimicrobial susceptibility testing of eight *Salmonella* isolates.

### **WHO SHOULD PARTICIPATE IN EQAS 2017?**

All national and regional reference laboratories which perform analysis on *Salmonella* and are interested in participating in an external quality assurance program are invited to participate.

We expect that all national and regional reference laboratories that attended WHO GFN Training Courses will participate in the EQAS.

The WHO GFN Regional Centers in cooperation with the EQAS Coordinator will evaluate the list of laboratories that sign up for EQAS 2017. Laboratories which signed up and received bacterial isolates in year 2016 but did not submit any result should provide a consistent explanation for this if they want to participate in 2017.

### **COST FOR PARTICIPATING IN EQAS**

There is no participation fee. Laboratories should, however, cover the expenses for parcel shipment if they can afford it. If FedEx has 'Dangerous Goods-service' in your country or if you have a DHL-account no, please provide your FedEx or DHL import account number (for import of UN3373 Biological Substance Category B) in the sign-up form or, alternatively, to the EQAS Coordinator (please find contact information below). We need this information at this stage to save time and resources. Participating laboratories are responsible for paying any expenses related to taxes or custom fees applied by their country.

## **HOW TO SIGN- UP FOR EQAS 2017**

This link will open a sign-up webpage: <http://eqas.food.dtu.dk/who/signup>

In this webpage, you will be asked to provide the following information:

- Name of institute, department, laboratory, and contact person
- Complete mailing address for shipment of bacterial isolates (no post-office box number)
- Telephone and fax number, e-mail address
- FedEx or DHL import account number (if available)
- Approximate number of *Salmonella* isolates annually serogrouped/serotyped
- Approximate number of *Salmonella* isolates annually tested for antimicrobial susceptibility
- Availability of ATCC 25922 *E. coli* reference strain
- Components of EQAS 2017 you plan to participate in
- Level of reference function in your country

If you experience any problem in the sign-up webpage, please try again a few days later. If problems persist, please contact the EQAS Coordinator Susanne Karlsmosse Pedersen: E-mail [suska@food.dtu.dk](mailto:suska@food.dtu.dk).

## **TIMELINE FOR SHIPMENT OF ISOLATES AND AVAILABILITY OF PROTOCOLS**

A number of different institutions will ship the bacterial isolates, and you will receive information concerning the institution shipping your parcel. The bacterial isolates will be shipped in ***October 2017***.

In order to minimize delays, **please send a valid import permit to the EQAS coordinator**. Please apply for a permit to receive the following: “UN3373, Biological Substance Category B”: eight *Salmonella* strains, and (for new participants performing antimicrobial susceptibility testing on *Salmonella*) one *Escherichia coli* reference strain.

Protocols and all relevant information will be available for download from the website <http://www.antimicrobialresistance.dk/233-169-215-eqas.htm>.

## **DEADLINE FOR SUBMITTING RESULTS TO THE NATIONAL FOOD INSTITUTE**

Results must be submitted to the National Food Institute (DTU Food) by **28<sup>th</sup> February 2018** through the password-protected website. An evaluation report will be generated upon submission of results. Full anonymity is ensured, and only DTU Food and the WHO GFN Regional Centre in your region will have access to your results.

**Deadline for sign-up for the WHO GFN EQAS 2017 is August 4<sup>th</sup>, 2017**

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Appendix 3, page 1 of 1

			Presumptive phenotype	Ampicillin AMP		Cefotaxime CTX		Synergy CTX:/CTX:CI	Cefoxitin FOX		Ceftazidime CAZ		Synergy CAZ:/CAZ:CI	Ceftriaxone CRO		Chloramphenicol CHL		Ciprofloxacin CIP	
WHO 2017 S-17.1	<i>Salmonella</i> Infantis	I 6,7:r:1,5	ESBL	>64	RESIST	>64	RESIST	synergy	4	SUSC	16	RESIST	synergy	>32	RESIST	<=8	SUSC	0.25	INTER
WHO 2017 S-17.2	<i>Salmonella</i> Havana	I 13,23:f,g:-	AmpC	<=1	SUSC	1	SUSC	no synergy	32	RESIST	4	RESIST	no synergy	0.12	SUSC	<=8	SUSC	0.06	SUSC
WHO 2017 S-17.3	<i>Salmonella</i> Enteritidis	I 9,12:g,m;-	-	4	SUSC	0.5	SUSC				1	SUSC		0.25	SUSC	16	INTER	0.06	SUSC
WHO 2017 S-17.4	<i>Salmonella</i> Rissen	I 6,7:f,g:-	ESBL	>64	RESIST	32	RESIST	synergy	8	SUSC	2	RESIST	synergy	64	RESIST	>128	RESIST	0.03	SUSC
WHO 2017 S-17.5	<i>Salmonella</i> Weltevreden	I 3,10:r;z6	-	<=1	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.03	SUSC	128	RESIST	0.03	SUSC
WHO 2017 S-17.6	<i>Salmonella</i> Schwarzengrund	I 4,12:d:1,7	-	2	SUSC	<=0.25	SUSC				<=0.5	SUSC		0.06	SUSC	<=8	SUSC	0.03	SUSC
WHO 2017 S-17.7	<i>Salmonella</i> Typhimurium	I 4,5,12:i:1,2	-	>64	RESIST	0.5	SUSC				<=0.5	SUSC		0.12	SUSC	64	RESIST	0.5	INTER
WHO 2017 S-17.8	<i>Salmonella</i> Kentucky	I 8,20:i;z6	Carbapenemase	>64	RESIST	>64	RESIST	no synergy	64	RESIST	>128	RESIST	no synergy	>256	RESIST	>128	RESIST	8	RESIST

			Presumptive phenotype	Colistin COL		Gentamicin GEN		Meropenem MER		Nalidixic acid NAL		Sulfonamides SMX		Tetracycline TET		Trimethoprim TMP		Trim/Sulfa SXT	
WHO 2017 S-17.1	<i>Salmonella</i> Infantis	I 6,7:r:1,5	ESBL	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>4	RESIST
WHO 2017 S-17.2	<i>Salmonella</i> Havana	I 13,23:f,g:-	AmpC	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	64	SUSC	4	SUSC	0.5	SUSC	0.12	SUSC
WHO 2017 S-17.3	<i>Salmonella</i> Enteritidis	I 9,12:g,m;-	-	2	SUSC	32	RESIST	0.06	SUSC	8	SUSC	>1024	RESIST	4	SUSC	<=0.25	SUSC	0.12	SUSC
WHO 2017 S-17.4	<i>Salmonella</i> Rissen	I 6,7:f,g:-	ESBL	<=1	SUSC	1	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.5	<i>Salmonella</i> Weltevreden	I 3,10:r;z6	-	<=1	SUSC	<=0.5	SUSC	0.06	SUSC	<=4	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.6	<i>Salmonella</i> Schwarzengrund	I 4,12:d:1,7	-	2	SUSC	<=0.5	SUSC	0.06	SUSC	8	SUSC	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.7	<i>Salmonella</i> Typhimurium	I 4,5,12:i:1,2	-	8	RESIST	<=0.5	SUSC	0.06	SUSC	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST
WHO 2017 S-17.8	<i>Salmonella</i> Kentucky	I 8,20:i;z6	Carbapenemase	<=1	SUSC	1	SUSC	1	RESIST	>128	RESIST	>1024	RESIST	>64	RESIST	>32	RESIST	>32	RESIST



# PROTOCOL for

serotyping and antimicrobial susceptibility testing of *Salmonella* test strains

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<b>1</b>	<b>INTRODUCTION .....</b>	<b>1</b>
<b>2</b>	<b>OBJECTIVES .....</b>	<b>2</b>
<b>3</b>	<b>OUTLINE OF THE EQAS 2017 .....</b>	<b>2</b>
<b>3.1</b>	<b>Shipping, receipt and storage of strains .....</b>	<b>2</b>
<b>3.2</b>	<b>Serotyping of <i>Salmonella</i> .....</b>	<b>2</b>
<b>3.3</b>	<b>Antimicrobial susceptibility testing of <i>Salmonella</i> strains and <i>Escherichia coli</i> ATCC 25922 .....</b>	<b>3</b>
<b>4</b>	<b>REPORTING OF RESULTS AND EVALUATION .....</b>	<b>7</b>
<b>5</b>	<b>HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE .....</b>	<b>7</b>

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## 1 INTRODUCTION

In 2000, the Global Foodborne Infections Network (formerly known as WHO Global Salm-Surv) launched an External Quality Assurance System (EQAS). The EQAS is organized by the National Food Institute, Technical University of Denmark (DTU Food), in collaboration with partners and Regional Sites in WHO GFN.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and the National Food Institute is responsible for the subcontractor's work.

The WHO EQAS 2017 includes serotyping and antimicrobial susceptibility testing of eight *Salmonella* strains and antimicrobial susceptibility testing of the *Escherichia coli* ATCC 25922 (CCM 3954) reference strain for quality control (QC).

The above-mentioned QC reference strain is included in the parcel only for new participants of the EQAS who did not receive it previously. The QC reference strain supplied is an original

CERTIFIED culture provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strain will not be included in the years to come. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual 'Subculture and Maintenance of QC Strains' available on the WHO Collaborating Centre website (see [www.antimicrobialresistance.dk](http://www.antimicrobialresistance.dk)).

## 2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of serotyping and antimicrobial susceptibility testing of enteric human pathogens, especially *Salmonella*. A further objective is to assess and improve the comparability of surveillance data on *Salmonella* serotypes and antimicrobial susceptibility reported by different laboratories. Therefore, the laboratory work for this EQAS should be done by using the methods routinely used in your laboratory.

## 3 OUTLINE OF THE EQAS 2017

### 3.1 Shipping, receipt and storage of strains

In October 2017 around 200 laboratories located worldwide will receive a parcel containing eight *Salmonella* strains. An *E. coli* ATCC 25922 reference strain will be included for participants who signed up to perform antimicrobial susceptibility testing (AST) and did not receive it previously. All provided strains belong to UN3373, Biological substance category B. Extended Spectrum Beta Lactamase (ESBL)-, AmpC- or carbapenemase-producing strains could be included in the selected material.

**Please confirm receipt of the parcel through the confirmation form enclosed in the shipment**

The *Salmonella* strains are shipped as agar stab cultures whereas the reference strain is shipped lyophilised. On arrival, the agar stab culture must be stored in a dark place at 2°C to 8°C. If receiving a lyophilized reference culture, store in a dark, cool place. The agar stab cultures must be sub-cultured and prepared for storage in your strain collection (e.g. in a -80°C freezer). This set of cultures should serve as reference if discrepancies are detected during the testing (e.g. they can be used to detect errors such as mis-labelling or contamination).

### 3.2 Serotyping of *Salmonella*

The eight *Salmonella* strains should be serotyped by using the method routinely used in the laboratory. Also serogroup results will be evaluated, therefore, if you do not have all the necessary antisera for a serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and

Weill, 2007. 9<sup>th</sup> ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

Please fill in information concerning the brand of antisera used for typing in the fields available in the database for entering results. In addition, we kindly ask you to report which antisera you think are required to complete the serotyping, if relevant.

### **3.3 Antimicrobial susceptibility testing of *Salmonella* strains and *Escherichia coli* ATCC 25922**

The *Salmonella* strains as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in your laboratory.

For reconstitution of the *E. coli* reference strain, please see the document 'Instructions for opening and reviving lyophilised cultures' on the WHO Collaborating Centre website (see [www.antimicrobialresistance.dk](http://www.antimicrobialresistance.dk)).

Testing of gentamicin susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* should not be reported as susceptible to aminoglycosides.

The breakpoints used in this EQAS for interpreting MIC results are in accordance with CLSI values (Table 1). Consequently, interpretation of MIC results will lead to categorization of strains into three categories: resistant (R), intermediate (I) and susceptible (S). In the evaluation report you receive upon result submission, you can find that obtained interpretations in accordance with the expected interpretation will be defined as 'correct', whereas deviations from the expected interpretation will be defined as 'minor' (I ↔ S or I ↔ R), 'major' (S interpreted as R) or 'very major' (R interpreted as S).

Please report the breakpoints that you routinely use in your laboratory for interpretation of antimicrobial susceptibility test results in the fields available in the database (or in the test forms).

**Table 1.** Interpretive breakpoint for *Salmonella* antimicrobial susceptibility testing

Antimicrobials	Reference value, MIC (µg/mL)			Reference value, Disk diffusion (mm)		
	Susceptible	Intermediate	Resistant	Resistant	Intermediate	Susceptible
Ampicillin, AMP	≤8	16	≥32	≤13	14-16	≥17
Cefotaxime, CTX*	≤1	-	>1	≤27	-	>27
Cefoxitin, FOX	≤8	16	≥32	≤14	15-17	≥18
Ceftazidime, CAZ*	≤1	-	>1	≤22	-	>22
Ceftriaxone, CRO*	≤1	-	>1	≤25	-	>25
Chloramphenicol, CHL	≤8	16	≥32	≤12	13-17	≥18
Ciprofloxacin, CIP	≤0.06	0.12-0.5	≥1	≤20mm (5µg) or ≤23mm (1µg)**	21-30mm (5µg) or (1µg)**	≥31mm (5µg) or ≥23mm (1µg)**
Colistin, COL***	≤2	-	≥4	Not applicable	Not applicable	Not applicable
Gentamicin, GEN	≤4	8	≥16	≤12	13-14	≥15
Meropenem, MER*	≤0.12	-	>0.12	<27	-	≥27
Nalidixic acid, NAL	≤16	-	≥32	≤13	14-18	≥19
Sulfonamides, SMX	≤256	-	≥512	≤12	13-16	≥17
Tetracycline, TET	≤4	8	≥16	≤11	12-14	≥15
Trimethoprim, TMP	≤8	-	≥16	≤10	11-15	≥16
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	≤2/38	-	≥4/76	≤10	11-15	≥16

Reference values used in this EQAS are according to CLSI (M100, 27<sup>th</sup> edition), with the following exceptions:

\* For the cephalosporins and meropenem, the application of the interpretative criteria is intended to indicate if the microorganism is a presumptive ESBL- or carbapenemase-producer. Reference values for the cephalosporins are according to CLSI M100 Table 3A. These interpretative criteria are also applied for *Salmonella* test strains for interpretation of AST results in this EQAS. Reference values for meropenem are based on epidemiological cut off values from [www.eucast.org](http://www.eucast.org).

\*\* The publication by Cavaco LM and Aarestrup FM (J. Clin. Microbiol. 2009. Sep;47(9):2751-8) provides the background for these interpretative criteria in the WHO GFN EQAS.

\*\*\* Reference values for colistin are based on CLSI M100 Table 2A-2. In the current EQAS these values should be applied for the interpretation of *Salmonella* AST results into the category as susceptible or resistant.

Concerning ciprofloxacin susceptibility tests, the applied breakpoints take into consideration mechanisms of resistance due to plasmid-mediated quinolone resistance genes (e.g. *qnr*-genes) and one-point-mutation in the gyrase gene.

Important notes: *beta-lactam resistance*

The following tests for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes are optional in relation to the current WHO GFN EQAS.

If choosing to participate in this component of the EQAS, all strains displaying reduced susceptibility to cefotaxime (CTX), ceftazidime (CAZ), and/or ceftriaxone (CRO) should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests. Reduced susceptibility to any of the above-mentioned antimicrobials indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype.

Confirmatory test for ESBL production requires the use of both cefotaxime (CTX) and ceftazidime (CAZ) alone, and in combination with a  $\beta$ -lactamase inhibitor (clavulanic acid). Synergy is defined either as i) by microbroth dilution methods or E-test; a  $\geq 3$  twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/Cl or CAZ : CAZ/Cl ratio  $\geq 8$ ) or ii) by disk diffusion; a  $\geq 5$  mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases can be performed by testing the bacterial culture for susceptibility to ceftiofur (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

Confirmatory test for carbapenemase production requires the testing of meropenem (MER). Reduced susceptibility to MER indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (available in The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015, EFSA Journal 2017;15(2):4694,212 pp (page 43).

The following summary of these recommendations indicate how the phenotypes should be categorized:

ESBL-phenotype:

- CTX or CAZ > 1 mg/L **AND**
- MER ≤ 0.12 mg/L **AND**
- FOX ≤ 8 mg/L **AND**
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

ESBL+AmpC-phenotype:

- CTX or CAZ > 1 mg/L **AND**
- MER ≤ 0.12 mg/L **AND**
- FOX > 8 mg/L **AND**
- Synergy for CTX : CTX/Cl and/or CAZ : CAZ/Cl

AmpC-phenotype:

- CTX or CAZ > 1 mg/L **AND**
- MER ≤ 0.12 mg/L **AND**
- FOX > 8 mg/L **AND**
- No synergy for CTX : CTX/Cl nor CAZ : CAZ/Cl  
 (note, presence of ESBLs is not excluded)

Carbapenemase-phenotype:

- MER > 0.12 mg/L  
 (note, presence of ESBLs or AmpCs is not excluded)

Other-phenotype:

- Not covered by any of the above categories **AND**
- CTX, CAZ, FOX, or MER > interpretative criteria as susceptible in Table 1 (i.e. exhibits reduced susceptibility)

No ESBL-, AmpC-, or carbapenemase:

- CTX, CAZ, FOX, and MER ≤ interpretative criteria as susceptible in Table 1 (i.e. exhibits susceptibility)

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but is not requested as part of this WHO GFN EQAS.



#### **4 REPORTING OF RESULTS AND EVALUATION**

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the web database. For entering your results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

**Results must be submitted no later than 28<sup>th</sup> February 2018.**

If you do not have access to the Internet, or if you experience difficulties in entering your results, please contact the EQAS Coordinator directly, explaining the issues that occur.

All results will be summarized in a report which will be publicly available. Individual results will be anonymous and will only be forwarded to the official GFN Regional Centre in your region.

We are looking forward to receiving your results.

**If you have any questions or concerns, please do not hesitate to contact the EQAS Coordinator:**

Susanne Karlsmosen Pedersen

National Food Institute, Technical University of Denmark

Kemitorvet, Building 204, DK-2800 Lyngby - DENMARK

Tel: +45 3588 6601

E-mail: [suska@food.dtu.dk](mailto:suska@food.dtu.dk)

Direct communication with the EQAS organisers must be in English.

#### **5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE**

Please carefully read these instructions before entering the web page. Remember that you need by your side the completed test forms and the breakpoint values you used.

In general, you can browse back and forth in the pages of the database. Always remember to save your input before leaving a page.

**WHO Collaborating Centre  
External Quality Assurance System (EQAS) 2017**



- 1) Enter the WHO Collaborating Centre website (from <http://www.antimicrobialresistance.dk>), then
  - a. Click on 'EQAS'
  - b. Click on the link for the interactive database (<http://eqas.food.dtu.dk/who>)
  - c. Write your username and password in lower-case letters and click on 'Login'.  
You can find your username and password in the letter following your strains.  
Your username and password will remain unchanged in future trials. Do not hesitate to contact us if you experience problems with the login.
- 2) Click on 'Materials and methods'
  - a. Fill in the fields relative to brand of antisera (very important because we would like to compare results obtained with different brands of antisera)
  - b. Fill in the fields relative to the method used for antimicrobial susceptibility testing
  - c. Enter the brand of materials, e.g. Oxoid
  - d. Fill in the field asking whether your institute serves as a national reference laboratory
  - e. In the comment field, report which antisera you think is required to complete your serotyping, if relevant
  - f. Click on 'Save and go to next page' – ALWAYS remember to save each page before leaving it!
- 3) In the data entry page 'Routinely used breakpoints'
  - a. Fill in the fields relative to the breakpoints used routinely in your laboratory to determine the antimicrobial susceptibility category. Remember to use the operator keys in order to show – equal to (=), less than (<), less or equal to (≤), greater than (>) or greater than or equal to (≥).
- 4) In the data entry pages '*Salmonella* strains 1-8',
  - a. SELECT the serogroup (O-group) from the drop-down list, DO NOT WRITE – Wait a few seconds – the page will automatically reload, so that the drop-down list in the field "Serotype" only contains serotypes belonging to the chosen serogroup.
  - b. SELECT the serotype from the drop-down list – DO NOT WRITE – wait a few seconds and you can enter the antigenic formula (e.g. 1,4,5,12:i:1,2)
  - c. Enter the zone diameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to (=), etc.
  - d. Enter the interpretation as R (resistant), I (intermediate) or S (susceptible)
  - e. If you performed confirmatory tests for ESBL production, select the appropriate result.
  - f. If relevant, fill in the field related to comments (e.g. which antisera you miss for complete serotyping)
  - g. Click on 'Save and go to next page'

If you did not perform these tests, please leave the fields empty



**WHO Collaborating Centre**  
**External Quality Assurance System (EQAS) 2017**



- 5) In the data entry page '*E. coli* reference strain':
  - a. Enter the zone diameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to (=), etc.
  - b. Click on 'Save and go to next page'
- 6) The next page is a menu that allows you to review the input pages and approve your input *and finally see and print the evaluated results*
  - a. Browse through the input pages and make corrections if necessary. Remember to click on 'save and go to next page' if you make any corrections.
  - b. Approve your input. Be sure that you have filled in all the results before approval, as **YOU CAN ONLY APPROVE ONCE!** The approval blocks your data entry into the interactive database, but allows you to see the evaluated results.
  - c. As soon as you have approved your input, an evaluation report will appear.
- 7) After browsing all pages in the report, you will find a new menu. You can choose 'EQAS 2017 start page', 'Review evaluated results' (a printer friendly version of the evaluation report is also available) or 'Go to WHO GFN homepage'.

**End of entering your data – thank you very much!**

# SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

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## 1 PURPOSE AND REFERENCES

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test (AST) results.

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

## 2 DEFINITION OF TERMS

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time.

## 3 IMPORTANT CONSIDERATIONS

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC.
- CLSI requires that QC be performed either on the same day or weekly (after QC-validation).
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides.

- Periodically perform colony counts to check the inoculum preparation procedure.
- Ideally, test values should be in the middle of the acceptable range.
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems.

#### 4 STORAGE OF REFERENCE STRAINS

##### Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen (alternatively, freeze dry).
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

##### Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

#### 5 FREQUENCY OF TESTING

##### Weekly vs. daily testing

Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.

- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

##### Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

If five acceptable QC results are available, no additional days of QC-testing are needed.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

# INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

*Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on <http://www.sci.muni.cz>.*

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

- Check the number of the culture on the label inside the ampoule
- Make a file cut on the ampoule near the middle of the plug (see Figure 1)
- Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
- Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
- Remove the pointed end of the ampoule into disinfectant
- Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
- Incubate the inoculated medium at appropriate conditions for several days
- Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

## Notes:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see <http://www.sci.muni.cz>)
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

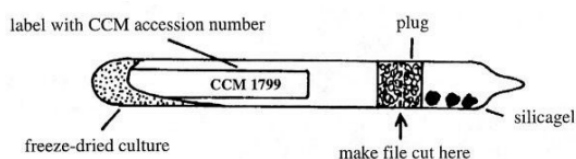


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on <http://www.sci.muni.cz>

National Food Institute  
Technical University of Denmark  
Kemitorvet  
Building 204  
DK - 2800 Kgs. Lyngby

Tel. 35 88 70 00  
Fax 35 88 70 01

[www.food.dtu.dk](http://www.food.dtu.dk)

ISBN: 978-87-93565-38-8